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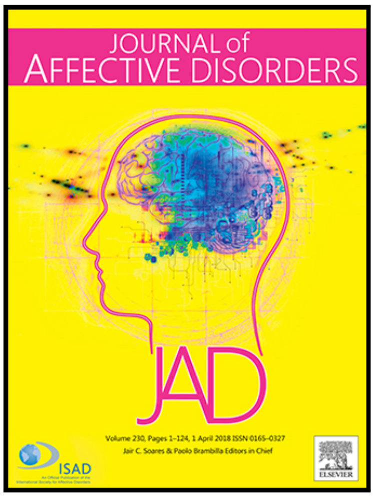

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Highlights

- The effects of maternal anxiety and depression in the 3rd trimester of pregnancy on fetal blink reaction to sound and light stimulation
- 20% increase in fetal eye blink for each unit increase in anxiety score
- 21% decrease in fetal eye blink for each unit increase in depression score
- Fetuses are affected differentially by maternal anxiety and depression
- Fetuses habituate to cross modal stimulation

Effects of maternal anxiety and depression on fetal neuro-development

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Running title: fetal blinking, maternal anxiety & depression

Background:

Fetal development is affected by maternal mental health with research indicating that maternal anxiety and depression are co-morbid; nevertheless differential effects on the fetus have been found. This study examines, prenatally, effects of maternal stress, anxiety and depression on fetal eye-blink reactions to experimental sound and light stimulation.

Methods:

Two groups of singleton fetuses (mean 32-weeks gestation) were examined using 4D ultrasound: a control group (N= 14, 7 female) with no stimulation and an experimental group (N=21, 13 female) exposed to experimental sound, light and cross-modal stimulation. For both groups ultrasound scans were performed and fetal eye-blink was assessed. Mothers completed the Hospital-Anxiety-and-Depression Scale and the Perceived-Stress Scale. Analysis was carried out using Poisson mixed effects modelling.

Results:

Fetal eye-blink rate during experimental stimulation was significantly and differentially associated with maternal mental health with a 20% increase of fetal eye-blink rate for each unit increase in anxiety score ($p=0.02$) and a decrease of 21% of eye blink rate for each unit of increase in depression score ($p=0.02$). Sound stimulation but not light stimulation significantly affected blink-rate with fetuses habituating to the stimuli ($p<0.001$).

Limitations:

Limitations are the relatively small number of fetuses and that a follow up after birth is essential to establish potential long-term effects.

Conclusions:

Of clinical importance is the finding that although fetuses are affected by maternal mental health in general here we demonstrate, using eye-blink-rate during stimulation as measure of neuro-development, that fetuses are differentially affected by maternal anxiety and depression with anxiety increasing and depression decreasing fetal reactivity significantly.

Keywords: fetuses; blinking; maternal anxiety; maternal depression; light stimulus; sound stimulus

1. Introduction

Maternal stress, anxiety and depression have been shown to directly affect fetal development, such as increasing arousal and influencing cortical processes (Kinsella & Monk, 2009). Specifically, maternal prenatal anxiety has been related to fetal movements (Kaitz, Mankuta, Rokem & Faraone, 2016) and to increased postnatal risk for neuro-developmental disorders (Kinsella & Monk, 2009; Glover, 2011; 2015; Sheridan, et al 2008, 2010). Some research indicates that both maternal anxiety and depression are co-morbid and one might influence the other. For example, Heron et al (2004) found that maternal antenatal anxiety predicted postnatal depression. Others (e.g., Beuke, Fischer & Dorwall, 2003; Pinto, Caldas, Nogueira-Silva & Figueiredo, 2017) claim that anxiety and depression need to be both measured on comparable scales in order to differentiate between the effects of anxiety and depression. In this paper we take the second approach.

One way in which the effect of maternal anxiety and depression on the fetus can be assessed is by examining eye blinking. Eye blink measures elicited through light and affective stimulation ex utero, relate to cognitive and emotional functions, which can be assessed in blood or urine by level of dopamine, a neurotransmitter involved in learning and goal oriented behaviour (e.g. Eckstein, Guerra-Carrillo, Miller Singley, & Bunge, 2017; Westbrook & Braver, 2016). Fetal eye blink reactions to light are dependent on the formation of rods and cones in the retina, which are known to be morphologically complete at about 22 weeks gestation, from which age fetuses are hypothesised to be sensitive to light (Kiuchi, Nagata, Ikeno & Terakawa, 2000).

Fetal eye blink rate is a measure of reaction to both external stimulation and emotional stimulation via maternal hormonal variation and to light and sound. Internal or hormonal stimulation is triggered by maternal anxiety, stress or depression and will also affect fetal eye blink rate (Ruiz-Padial, Sollers,Vila, & Thayer, 2003).

Fetuses, although blinking spontaneously, do so at a much reduced rate without stimulation. In contrast the increased fetal eye blink rate to external stimulation such as light, vibration or sound, reflects neuro-maturation of the fetus (Kisilevsky & Muir, 1991; Andonotopo & Kurjak, 2006; Hata, 2010). Regarding cross model stimulation, there is some research indicating that sound modulates responses to light in rabbits at the neuronal level in the primary visual cortex (Polyanskii, Alymkulov, Evtikhin & Chernyshev, 2013). Hence, we would expect variations in fetal blink reactions to cross modal light and sound stimulation versus only light or only sound stimulation.

In human fetuses, eye-blinks have been observed both without external stimulation (Petrikovsky, Kaplan & Holsten, 2003) and in response to vibroacoustic stimulation (Hata, Kanenishi, Akiyama, Tanaka & Kimura, 2005). In an observational study using 4D ultrasound assessment fetal eyelid movement was observed in three of 17 fetuses (17.6%) and a double blink was identified in one fetus at 38 weeks (Hata et. al., 2005). In a study of 227 fetuses, Birnholz & Benacerraf (1983) reported that blink-startle response to vibroacoustic stimulation could be detected in fetuses as young as 24 weeks and was consistently found after 28 weeks. They further reported that blinking frequency increases with fetal age. Using two-dimensional (2D) sonography, Petrikovsky et. al. (2003) detected fetal eye-blinking in 16 out of 18 fetuses aged between 33-42 weeks gestation. In a subsample of 6 fetuses, vibroacoustic stimulation was applied. In all stimulated fetuses they observed an increase in fetal blinking. They reported that the "no stimulation" baseline of 0.10 blinks per minute increased post stimulation to 0.26 blinks per minute. These results support the claim that eye-blink increases during heightened arousal (Stern, Walrath & Goldstein, 1984).

A number of studies have indicated that fetal reactions are traceable to the frontal cortex indicating that fetuses are at some level conscious of the stimulation received (Lagercrantz, 2014). A study using functional Magnetic Resonance Imaging (fMRI) on pregnant women with normal, healthy singleton fetuses over 36 weeks gestational age reported significant evoked cortical potentials in the frontal cortex of human fetuses in response to artificial light stimulation (Fulford et al., 2003). Using real-time ultrasonography, in one study 56 healthy fetuses aged between 36 and 40 weeks gestation were examined

(Kiuchi, et al., 2000). A photographic flash, applied directly to the maternal abdomen, resulted in an increase in rapid eye movements, body movements and fetal heart rate, indicating that fetuses respond to light stimulation. Furthermore, fetuses react to specific configurations of light stimulation (Reid et.al., 2017).

Despite studies finding specific fetal response to light stimulation, not all literature is in agreement. Two studies using a Magnetoencephalography (MEG) scan (Matuz et. al., 2012; Sheridan et. al., 2008), found that for a sizable majority of fetuses there was no visual evoked potential in response to flashing light stimulation. They reported that only 11 out of 25 fetuses (Sheridan et al., 2008) and 15 out of 33 fetuses (Matuz, et al., 2012) showed any indication of cortical response. This might be the result of differences in maternal tissue thickness preventing light penetrating the womb and reaching the fetal eye.

How much light penetrates through maternal tissue depends on the tissues properties, such as water, blood, fat and collagen content (Jacques, 2013). For example, fat increases light scattering (Jacques, 2013) therefore the thicker the layer of fat, the lower light transmission through the tissue. Del Giudice (2011) suggests for fetuses a minimum threshold of 10 lux at the fetal eye would be sufficient for prenatal visual experience. To establish whether it is possible for this much light to reach the uterine cavity, Del Giudice (2011) tested light transmission in vitro through skin, adipose tissue, and muscle and reported that light penetrated all of these with sufficient quantities to enable visual perception; light levels in the womb were found to be between 0.1- 1.0% of the external illuminance. Del Giudice's (2011) findings suggest, given bright enough external light, it is possible for there to be sufficient illumination in the womb interior for the fetus to react to light stimulation.

In summary, in various studies, fetal eye-blink reactions have been reported to be indicators of cognitive processing or fetal arousal level. However, there are no studies to date examining prenatally the effects of both maternal anxiety and depression alongside light and sound stimulation. Hence, it is unclear whether eye-blink frequency is a response to maternal mood or the external stimulus or both.

As it has been shown that fetuses respond with changed level of arousal when exposed to maternal anxiety, stress and depression, we tested a sample of non-clinical healthy sample of pregnant women for the effects of maternal levels of anxiety and depression on rate of fetal eye blink. Given previous research on fetal reactions to sound (Reissland, Francis, Buttenshaw, Austen & Reid , 2016a) and light (Reid et al 2017) we expected that during experimental stimulation fetuses would react with changed eye blink rates, but also expected that maternal anxiety and depression would affect eye-brink rates.

2. Methods

2.1 Participants

Participants were a opportunity sample of 35 healthy pregnant women, not on medication with healthy singleton fetuses (20 girls), as assessed at their 20 week anomaly scan and their birth record, with mean gestational age at scan 32.2 weeks (range 31.1 to 33.7 weeks); and with mean gestational age at birth of 40.05 weeks (range 37.0 to 42.5 weeks). Participants were Caucasian from the North East of England and scanned either at the antenatal unit of the James Cook University Hospital, Middlesbrough, UK or the Middlesbrough BabyBrite Clinic by experienced sonographers.

All mothers gave informed written consent. The fetuses were either in a control group (N= 14, 7 female; mean gestational age 32.1 weeks, range 31.1-33.1 weeks) not receiving any experimental stimulation or an experimental group (N=21, 13 female; mean age 32.3 weeks, range 31.5-33.7 weeks) receiving light and sound stimulation. All mothers came from the same catchment area. The stimulated and non-stimulated groups were equivalent in terms of social class with the group exposed to light and sound stimulation including similarly skilled participants (50% Administrative, 40% skilled manual and 10% semi-skilled) as the group not receiving light and sound stimulation (46.2% Administrative, 30.8% skilled manual and 23% semi-skilled).

2.2 Ethics

This study was conducted in accordance with the Declaration of Helsinki. Ethical permission for the control group was granted by the County Durham and Tees Valley 2 Research Ethics Committee (REC Ref: 08/H0908/31) and the research and development department of James Cook University Hospital, as well as the Durham University (Department of Psychology ethics committee). Ethical permission for the experimental study was granted from Durham University (Department of Psychology Ethics Committee, N Reissland 14/51 by Durham University). All mothers gave informed written consent.

2.3 Stimuli

2.3.1 Light:

The light source consisted of a red LED with a centre wavelength of 640nm. The output was measured to be 0.6 lumens, on the participant's abdomen, with a surface area of 78.54mm2, giving an external light illuminance of 7638.44 lux. Light intensity received by the fetus depends on the intervening thickness of abdominal muscle, placenta and fat. We assessed the light intensity received by the fetus by basing our approach on work by Del Giudice (2011). His approach was as follows. First, given muscle thickness m and the thickness of adipose tissue a (in mm), a transmission coefficient T gives the ratio between the internal illuminance received by the fetus and the external illuminance from the light source. His formula for the transmission coefficient is given by

$$\log_{10}(T) = -0.942 - 0.058\,m - 0.032a$$

We noted, however that this formula fails to include placental tissue. This tissue is more translucent than muscle and less translucent than adipose tissue, and can also be clearly differentiated on ultrasound scans. We therefore adopted a mid-point coefficient of 0.045 for placental tissue, which lies halfway between the high value of 0.058 for muscle and the low value of 0.032 for adipose tissue. The formula then becomes:

$$\log_{10}(T) = -0.942 - 0.058\,m - 0.045p - 0.032a$$

where p is the thickness in mm of placental tissue.

Finally, we estimated the internal illuminance for each fetus by multiplying the external illuminance (7638.45 lx) by T.

2.3.2 Sound stimuli:

The auditory stimuli, based on the fact that the fundamental frequency (F0) of vowels are well transmitted to the fetus (e.g. Querleu, Renard, Boutteville & Crepin, 1989), we used the sounds MA (/maː/in the International Phonetic Alphabet), ME(/miː/ in the International Phonetic Alphabet), MO (/mɒː/ in the International Phonetic Alphabet) and white noise. The first three sounds were spoken by a female voice, were 0.40 s in length and were repeated 8 times with 0.80 s of silence between each presentation. Each cluster of eight was repeated for at least one minute. For white noise, the sound was played continuously for the same length in time as a single vocal sound. All sounds were played through a Sansa Clip portable MP3 device connected to a Anker portable speaker at frequencies between 0Hz and 11kHz, with most outputs at 0.6-1.6kHz and 2.6-3kHz regions. These frequencies are audible to fetuses from 29 weeks gestation (Gerhardt & Abrams, 2000; Reissland, et. al., 2016a) but produce a safe level of sound which will not harm fetal hearing (Krueger, Horesh & Crossland, 2012). Fetuses were exposed to sound only, sound and light and light only conditions. The loudspeaker, fitted with the light, was held approximately 1cm from the mother's abdomen. The sound and light condition consisted of the sound and a flashing light which flashed in time and to the same rhythm as the recorded sound. The light only condition consisted of a rhythmic flashing light similar to the "sound and light" condition and was again presented for at least one minute.

2.4 Ultrasound recording:

All groups received a 4D ultrasound scan lasting 15-20 minutes. 4D ultrasound scans were conducted and recorded for offline analysis using a GE Voluson P8 probe RAB2-5-RS/OB Expert Ultrasound System or the GE 8 Expert Ultrasound System using a GE RAB4-8L Macro 4D Convex Array Transducer. All ultrasound scans followed British Medical Ultrasound Society (BMUS, 2009) guidelines concerning the temperature and mechanical indices. The scanning took place with mothers lying in a darkened room on their back or on their side, depending on the position of the fetus and comfort of the mothers. Fetal head position was established in 2 D mode and then the sonographer switched to 4 D mode during which either the stimuli were presented to the fetus in the experimental group or the fetuses were simply observed in the control group.

2.5 Coding of fetal eye blinking

Coding was done offline using the Observer system (Noldus et al, 2000). The control fetuses scanned using 4D ultrasound but not receiving any additional stimulation, were coded for eye blink frequency. In the experimental group each fetus was presented with the randomly assigned experimental conditions and their eye blinks were coded during the stimulation. In addition for the experimental group womb thickness (and the proportions which were muscle, fat and placenta) was calculated from the ultrasound scan.

The 4D scans were coded using the Fetal Observable Movement System (FOMS) (Reissland, Francis & Buttanshaw, 2016b); an objective coding system based on anatomical muscle movements in the human fetal face. Eye-blink was defined as a lid movement from closed eyes to open eyes and closing again. Open eye was determined when the pupil and cornea were visible and there was movement of the eyelids. Anonymized clips were coded for eye blink frame by frame.

Coders were blind to condition. For each observation period where possible 60 seconds of scan was coded. The coding was not necessarily consecutive, depending on when the fetus' eyes were visible, starting with the first moment the eyes were codable.

When the scan became un-codable, for example when the eyes were obscured by the fetuses' hand, coding was suspended and restarted as soon as the eyes became visible again. The length of codable scan for each observation period was recorded allowing rates to be determined. Variations in length of coding were statistically controlled. Reliability tests were carried out by independent coders on 12.6% of observations for which the mean Cohen's Kappa was 0.93 with a range of 0.67- 1.00.

2.6 Measurement of maternal anxiety, depression and stress

In order to assess the emotional state, pregnant women completed the Hospital Anxiety (7 items) and Depression Scale (7 items) (HADS) (Zigmond & Snaith, 1983), and the Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983) consisting of 10 items. Mothers of fetuses (mean gestational age 32 weeks), serving as the control group also completed the HADS and PSS. Given that the HADS has been found to be an adequate measure of both anxiety and depression in comparison to State and Trait Anxiety Inventory (STAI) and the Beck Depression Inventory (BDI) (Mogg, Millar, & Bradley, 2000), we used the HADS for our measure.

2.7 Statistical Analysis

The analysis proceeded in two stages. For the first analysis, we compared the effect of experimental (sound and /or light) versus control (no sound or light) treatment conditions, examining the relative rate of eye blinks in the two conditions. We also compared the stress, anxiety and depression scores using t-tests. Significance was assessed by Wald test p-values.

For the second stage of the analysis, we examined the 21 fetuses in the experimental group, allowing us to focus in more detail on the various forms of stimulation, and also to take account of the internal light intensity received by the fetus. This was a repeated measures design, with multiple eye blink rates recorded for each fetus under each experimental condition. Additionally the observed scan length varied between experimental conditions and from fetus to fetus. Here, therefore, a mixed effects Poisson log-linear model was fitted (see Reissland, Francis & Mason, 2012; Reissland, Francis, Aydin, Mason & Schaal, 2014; Reissland et. al., 2016a), using number of eye-blinks as the dependent variable with an offset of log codable scan length (thus modelling eye blink rate) and with explanatory variables of sound type, light presence, experimental condition order (assessing habituation), light intensity at the fetus, gender of fetus, and depression, anxiety and stress, and with a random effect assessing between fetus variability. This model was fitted using the glmer function in the lme4 library in R (Bates, Maeschler, Bolker & Walker, 2015). Significance was again assessed by Wald test p-values on the parameter coefficients.

3. Results

3.1 Experiment vs control analysis

All of the fetuses in the experimental group had some eye blinking recorded during the scan, compared to only 11 of the 14 controls. In the experimental group the mean rate of eye-blink was 2.2 blinks per minute compared to 0.23 blinks per minute in the control group; a near ten- fold difference ($p<0.001$). There were no differences between the control and experimental groups in terms of maternal mental health characteristics. The mean stress score for controls was 13.71 and 11.67 for the experimental group ($t = 1.032$; $p=0.35$); for anxiety the corresponding means were 5.43 and 5.62 ($t=-0.187$; $p=0.85$) and for depression the means were 4.07 and 3.38 ($t=0.690$; $p=0.48$).

3.2 Analysis of experimental group

Table 1 shows the raw means (and standard deviations) of the eye blink rates per minute for each experimental condition as well as the number of eye blinks in the non-stimulated group. We can identify that there appear to be differences between experimental conditions in blinking rate, but these means are uncorrected for light intensity and maternal mental health and also do not account for the repeated measures nature of the design. The distribution of eye blink rate was also observed to be highly skewed, suggested that normality assumptions would be violated. We therefore proceed to the second stage of analysis.

Table 2 shows the regression coefficients of the Poisson mixed effects analysis, together with estimate of the between-fetus random effect with its 95% confidence interval. The estimates are on the log scale, and it is necessary to exponentiate the estimates to interpret the effects, which are multiplicative on the mean rate. These exponentited values are given in column 3 of Table 2. We first note the estimate of the between fetus variability of $\hat{\sigma}$=0.513 (with 95% confidence interval of 0.341to 0.784) which indicates a large and significant variability between fetuses. Some fetuses naturally blink more than others at 32 weeks gestation even after controlling for experimental factors.

In examining the effect of sound, we observe that the estimates for “white noise” are highly positive and significant ($p<0.001$) when compared to the reference “no sound” group. The estimate is 1.168, which, when exponentiated, gives a multiplicative effect on the eye blink rate of 3.216- a trebling of the white noise blink rate over the no sound group. The estimate of the MA sound condition was also significant ($p=0.018$), which doubles the rate (2.003) over the no sound group. The effect of light however is not significant. Both the presence of light ($p=0.207$) and the light intensity ($p=0.069$) were not significant.
Moving on to the effect of maternal mental health, we notice that both the HADS depression ($p=0.020$) and anxiety ($p=0.023$) scores show significant effects. The effects are large, with a unit increase in HADS anxiety score associated with a 20% increase in eye blink rate, and a unit increase in HADS depression score associated with a 21% decrease in eye blink rate. Stress of the mother and gender of the fetus however were not significant in the analysis once anxiety and depression had been controlled for..

Finally, we noted a strong order effect, with a sudden decline (halving) in eye blinking rate in the fourth experimental condition ($p<0.001$) compared to the first condition. This probably indicates a habituation effect.

4. Discussion

In the current study we tested fetal blink response when exposed to light, sound and cross modal stimulation, additionally measuring the effects of maternal emotional state, including self-reported stress, anxiety and depression. We found that fetuses varied their eye blink rate differentially depending on maternal anxiety and depression with an increased rate when mothers showed elevated levels of anxiety and decreased rate when mothers reported elevated rates of depression. This result concurs with other recent research. O'Conner, Heron & Glover (2002) found that in fetuses of 32 weeks gestation maternal anxiety was a significant predictor of behavioural and emotional problems at age 4 and 7. Additionally, van den Bergh, Mulder, Mennes, & Glover (2005) in a review of maternal anxiety and stress conclude that antenatal anxiety and stress affected neuro behavioural development postnatally. A possible mechanism which has been suggested by van den Bergh et al (2005) is that maternal mental health affects the programming of the HPA-axis and prefrontal cortex. This could explain why fetuses of anxious or depressed mothers react with different rates of eye blink to experimental sound and light stimulation compared with fetuses of mothers showing less anxiety and depression.

Furthermore, although anxiety and depression are correlated that was taken account of in the Poisson log-linear model. Regarding the fact that even if conditions are comorbid in the mother they can have different effects on the fetus and child, Tiemeier (2017) wrote that although anxiety in pregnancy is being recognized as a risk factor for postnatal neurodevelopmental variations depression seems to have less of an effect on the fetus. O'Connor et al (2002) found that antenatal maternal anxiety in late pregnancy but not antenatal depression was independently associated with behavioral/emotional problems when the children were 4 years old. Furthermore, Pinto et al (2017) reported that although depression and anxiety were comorbid in their sample only anxiety had a significant effect on both fetal- and birth- weight. Comparable results have been found by Waters et al (2014) in relation to skin conductance data of children of depressed and anxious mother, where children of depressed mothers showed smaller skin conductance responses compared to children of anxious mothers who showed larger skin conductance responses. Hence we argue in common with other researchers, that although anxiety and depression are co-morbid they have different effects on the offspring and we show in the current study that this is apparent prenatally.

It is important to note that in the experimental group in the current study the mean rate of eye-blink was 2.17 blinks per minute compared to 0.23 blinks per minute in the control group. This contrasts with Petrikovsky's et al. (2003) findings who reported substantially lower rates: a no stimulation baseline of 0.10 blinks per minute which increased post stimulation to 0.26 blinks per minute. However, in contrast to our sample, who had been found to be healthy at their 20 week anomaly scan, the sample of women observed by Petrikovsky et al. (2003) consisted of women had been referred for sonographic assessment because of suspected problematic pregnancies including decreased fetal movements, which might be the reason for the relatively low blink frequency observed in their sample. This interpretation is supported by research indicating that lack of fetal habituation to stimulation has been found to be related to maternal depression (Allister, Lester, Carr, & Liu, 2001) or stress (Sandman, Glynn, Wadhwa, Chicz-DeMet, Porto, & Garite, 2003). One limitation of the current study is that a follow up of fetuses after birth is essential to establish the long-term effects.

Light intensities reaching the fetal eye were sufficient to be perceived by fetuses. For example, 0.267 lux , the amount of light from a full moon overhead (Schlyter, 2017) was exceeded in all but one of the fetuses in the experimental group. Although some studies using brighter light intensities have been unsuccessful in producing fetal response (Matuz et al., 2012), intensities as low as this have elicited fetal brain activation (Fulford et al., 2003). Interestingly, in our study the level of light intensity was controlled for but did not affect fetal blink rate.

Fetuses were exposed randomly to various sound stimulations (MA, ME, Mo and white noise) and the fact that we noted a strong order effect, with a sudden decline in eye blinking rate in the fourth experimental condition ($p<0.001$) is indicative of habituation to stimulation and supports the idea that fetal eye blink rate reflects neuro-maturation of the fetus (Kisilevsky & Muir, 1991; Andonotopo & Kurjak, 2006; Hata, Daib, & Marumoc, 2010). Habituation to repeated stimulation, which is the most basic form of learning, is indicative of neuro maturation. Indeed, Leader (2016) reports that by 30 weeks gestation all fetuses in their study habituated to vibro-acoustic stimulation (VAS). Matuz et al (2012) report that fetuses, although not as responsive to visual habituation compared with neonates, habituated to visually presented light flashes. Variation in fetal ability to habituate has been associated with maternal mental health such as depression and anxiety. For example, one study found that fetuses of depressed and anxious mothers habituated more quickly and more fully to a vibro-acoustic stimulus placed on the maternal abdomen than fetuses unexposed to maternal depression and anxiety (e.g. Dieter, Emory, Johnson, & Raynor, 2008). Furthermore, spontaneous eye blink rate, is a non-invasive measure of central dopamine activity (Barbato et al 2012). Grigore, Gafitanu, Socolov, Grigore, Nemeti, G., & Micu, R. (2018) argued that blinking is an important parameter for fetal brain functional development and is regulated by the dopamine system. This might therefore explain variations in blinking observed as well as the fact that fetuses seemed to habituate and show a significantly reduced rate of blinking in the fourth (randomly presented) condition.

In conclusion, the results of this study demonstrate that fetuses appear to be sensitive to levels of anxiety and depression of the mother, and suggest that the use of eye-blink to study fetal cognition needs to control for maternal levels of anxiety and depression. Furthermore, given that fetal blink rate was affected by maternal emotional state rather than purely by the light and sound stimulation it can be concluded that increased rates of fetal eye blink are indicative of sensitivity of the fetus brought about by maternal anxiety. These findings highlight the need for more research on fetal perception and reaction to light and sound, but care needs to be taken of the intervening amounts of amniotic fluid, maternal fat, muscle, and placenta in order to assess the amount of light received by the fetal eye. A limitation of this study was the small sample size and future research, which could not be done with this relatively small sample, will help to clarify the age and conditions under which the rate of fetal eye blink is an indicator of the effects of maternal anxiety, depression and/or fetal neuro development.

Contributors
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Emma Reames
John Girkin

Authors statements:

- **N Reissland**: Study design, Sound stimuli design, Conduct of experiment, statistical analyses Writing up of the study
- **Suzanne Froggatt** : Coding of data, preparation of SPSS file, Drafts commented on
- **Emma Reames**: Study design, Coding of data, Write up of study, Drafts commented on
- **John Girkin**: designed the light stimulus including calculation of the light emitted as well as the apparatus to deliver light and sound, Drafts commented on
- All authors have approved the final article.

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'Declarations of interest: none'.

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Table 1: Mean eye blink rate (standard deviation in brackets) in the non-stimulated group of fetuses and the stimulated group of fetuses exposed to "Ma,Me, Mo" sounds and "White noise" with and without light stimulation

Mean eye blink rate per minute (standard deviation in brackets)		
No stimulation (Control) 0.23 (0.25)		
sound stimulation	light stimulation	no light stimulation
No sound	1.32 (1.65)	
Ma	2.19 (2.31)	2.22 (2.46)
Me	1.86 (2.11)	1.96 (1.68)
Mo	2.41 (2.94)	1.93 (2.28)
White noise	2.83(2.40)	3.11 (4.03)

Table 2. Parameter estimates of the Poisson mixed effects regression model.

Variable	Estimate	Std. error	Exp(estimate)	z-value	P-value	
Fixed effects:						
(Intercept)	-4.529	0.482				
Gender	-0.295	0.291	0.744	-1.016	0.310	
MA sound	0.695	0.293	2.003	2.371	0.018	*
ME sound	0.522	0.294	1.685	1.775	0.076	.
MO sound	0.567	0.296	1.763	1.913	0.056	.
White noise	1.168	0.310	3.216	3.774	<0.001	***
Light intensity	0.012	0.007	1.012	1.818	0.069	.
Light presence	-0.211	0.167	0.810	-1.263	0.207	
Presentation order 2	0.018	0.130	1.018	0.137	0.891	
Presentation order 3	-0.113	0.144	0.893	-0.783	0.433	
Presentation order 4	-0.745	0.172	0.475	-4.323	<0.001	***
HADS_Anxiety	0.185	0.080	1.203	2.322	0.020	*
HADS_Depression	-0.232	0.102	0.793	-2.278	0.023	*
PSS	0.034	0.033	1.035	1.025	0.305	
Random effect Between fetus	$\hat{\sigma}$=0.513 (95% CI=0.341, 0.784)					